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**14. ABSTRACT**
In this Project, we took a forward engineering approach to understanding and controlling gene expression. Specifically, we developed a theory of gene networks that will execute a prescribed series of cellular functions. By designing the gene networks ourselves, we can allow only simple interactions and thus avoid the enormous complexity that biology has developed over years of evolution. This project involved both theoretical and experimental work.

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# Final Report



GRANT #: N00014-99-1-0554

PRINCIPAL INVESTIGATOR: Dr. James J. Collins (jcollins@bu.edu)

PI INSTITUTION: Boston University

GRANT TITLE: Design and Construction of Genetic Applets

AWARD PERIOD: 1 April 1999 - 31 May 2003 (49 months)

## OBJECTIVE:

To model, design and construct synthetic gene networks (genetic applets) which can be programmed into DNA and delivered into cells to execute coordinated sequences of cellular functions while responding to intracellular and extracellular signals.

## APPROACH:

In this project, we took a forward engineering approach to understanding and controlling gene expression. Specifically, we developed a theory of gene expression that can be used to design gene networks that will execute a prescribed series of cellular functions. By designing the gene networks ourselves, we can allow only simple interactions and thus avoid the enormous complexity that biology has developed over years of evolution. This project involved both theoretical and experimental work.

## ACCOMPLISHMENTS:

We constructed a genetic toggle switch - a synthetic, bistable gene regulatory network - in *Escherichia coli*, and we developed a simple theory that predicts the conditions necessary for bistability. The toggle is constructed from any two repressible promoters arranged in a mutually inhibitory network. It is flipped between stable states using transient chemical or thermal induction and exhibits a nearly ideal switching threshold. We also explored the effects of noise on gene expression. We considered a single network derived from bacteriophage lambda, and constructed a two-parameter deterministic model describing the temporal evolution of the concentration of lambda repressor protein. We showed how additive and multiplicative external noise can be used to regulate expression. In the additive case, we demonstrated the utility of such control through the construction of a protein switch, whereby protein production is turned "on" and "off" using short noise pulses. In the multiplicative case, we showed that small deviations in the transcription rate can lead to large fluctuations in the production of protein, and we described how these fluctuations can be used to amplify protein production significantly. We developed a mathematical model of an autocatalytic single-gene switching network. We also designed and constructed an autocatalytic single-gene switching network in *E. coli*. We conducted extensive switching experiments with this system. In addition, we designed and constructed a eukaryotic genetic toggle switch. We conducted preliminary experiments on this system. Moreover, we developed a

mathematical model of an engineered intercellular signaling system for synchronizing synthetic gene oscillators. We showed analytically and computationally that such a system can be used to synchronize synthetic gene oscillators. We also developed a model for a synthetic gene network for entraining and amplifying cellular oscillations.

## CONCLUSIONS:

The experiments on the autocatalytic single-gene network showed that protein destabilization leads to the existence of two expression states, as predicted by the model incorporating the known molecular reactions and biochemical rates. The observed population distributions and coefficients of variation were in striking quantitative agreement with those predicted by the stochastic version of the model. This work demonstrates that theoretical models, coupled with isolated gene circuits, can be utilized to establish a framework for deducing the dynamics of gene regulation and the development of engineered cellular control. We also designed a synthetic gene network in E. coli that acts as a relaxation oscillator, and uses an intercell signaling mechanism to couple the oscillators and induce synchronous oscillations. We modeled the system and showed that the proposed coupling scheme leads to synchronous behavior across a population of cells. We provided an analytical treatment of the synchronization process, the dominant mechanism of which is "fast threshold modulation." We also developed a model for a synthetic gene oscillator and considered the coupling of the oscillator to a periodic process that is intrinsic to cells. We investigated the synchronization properties of the coupled system, and showed how the oscillator can be constructed to yield a significant amplification of cellular oscillations. We reduced the driven oscillator equations to a normal form, and analytically determined the amplification as a function of the strength of the cellular oscillations. The ability to couple naturally-occurring genetic oscillations to a synthetically designed network could lead to possible strategies for entraining and/or amplifying oscillations in cellular protein levels. We also found that we could obtain bistable switching dynamics with the eukaryotic genetic toggle switch. Our modeling work on the intercellular signaling system showed that it should be possible to engineer such a system based on bacterial quorum-sensing mechanisms, and to use such a system to synchronize synthetic gene oscillators.

## SIGNIFICANCE:

As a practical device, the toggle switch forms a synthetic, addressable cellular memory unit and has implications for biotechnology, biocomputing and gene therapy. In addition, the reasonable agreement between the toggle theory and experiment suggests that the theoretical design of complex and practical gene networks is a realistic and achievable goal. Furthermore, the genetic toggle switch forms the basis for a forward engineering approach to the study of gene expression. Such an approach may be more effective than the reverse engineering approach more typically used in cellular biology because it permits the complete manipulation of all elements in the system. The enormous complexities of natural gene networks can be engineered out of the toggle switch and future experimental devices. Thus, synthetic gene networks, which serve as highly simplified, highly controlled models of natural gene networks, can be used to test and refine a more general, quantitative theory of gene regulation. Our results concerning the effects of noise on gene regulation suggest that an external noise source could be used as a switch and/or amplifier for gene expression.

Synthetic gene networks can be viewed as a first step towards logical cellular control, whereby biological processes can be manipulated or monitored at the DNA level. From the construction of a simple set of genetic building-block circuits (e.g., toggle switches, oscillators, etc.), one can imagine the design and construction of integrated biological circuits capable of performing increasingly elaborate functions. An integrated biological circuit could, like electronic control circuits, possess data-processing and storage circuitry, as well as input/output components necessary for sensing and affecting its environment. Ultimately, synthetic gene circuits encoded into DNA, might be "downloaded" into cells creating, in effect, a "wet" nano-robot. These cellular robots could be utilized for a variety of functions, including in vivo biosensing, autonomously synthesizing complex biomaterials, executing programmed cell death, and interfacing with microelectronic circuits by transducing biochemical events to and from the electronics.

**PATENT INFORMATION:**

1. Gardner TS and Collins JJ. "Bistable Genetic Toggle Switch." Patent pending.

2. Collins JJ, Gardner TS, di Bernardo D, Yeung MKS and Tegner J. "Systems and Methods for Reverse Engineering Models of Biological Networks." Patent pending.

**AWARD INFORMATION: (received by Project PI during the entire award period)**

1. Fellow, Institute of Physics.

2. Engineering in Medicine and Biology Society (EMBS) Early Career Achievement Award.

3. Metcalf Cup and Prize for Excellence in Teaching - the highest teaching honor awarded by Boston University.

4. Sanctae Crucis Award for Outstanding Young Alumnus, College of the Holy Cross.

5. Fellow, American Institute for Medical and Biological Engineering (AIMBE).

6. Fellow, American Physical Society.

7. Technology Review's TR100: the top 100 young innovators who will shape the future of technology.

**PUBLICATIONS IN REFEREED JOURNALS: (Cumulative for the project)**

1. Gardner TS, Cantor CR and Collins JJ. Construction of a genetic toggle switch in Escherichia coli. Nature 403: 339-342 (2000).

2. Hasty J, Pradines J, Dolnik M and Collins JJ. Noise-based switches and amplifiers for gene expression. Proceedings of the National Academy of Sciences USA 97: 2075-2080 (2000).

3. Gardner TS and Collins JJ. Neutralizing noise in gene networks. Nature 405: 520-521 (2000).

4. Hasty J, Isaacs F, Dolnik M, McMillen D and Collins JJ. Designer gene networks: towards fundamental cellular control. CHAOS 11: 207-220 (2001).

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7. McMillen D, Kopell N, Hasty J and Collins JJ. Synchronizing genetic relaxation oscillators with intercell signaling. Proceedings of the National Academy of Sciences USA 99: 679-684 (2002).

8. Hasty J, Dolnik M, Rottschafer V and Collins JJ. A synthetic gene network for entraining and amplifying cellular oscillations. Physical Review Letters 88: 148101 (2002).

9. Hasty J and Collins JJ. Translating the noise. Nature Genetics 31: 13-14 (2002).

10. Hasty J, McMillen D, and Collins JJ. Engineered gene circuits. Nature 420:224-230.

11. Isaacs F, Hasty J, Cantor C, Collins JJ. Prediction and measurement of an autoregulatory genetic model. Proceedings of the National Academy of Sciences USA 100: 7714-7719 (2003).

12. Gardner T, di Bernardo D, Lorenz D, Collins JJ. Inferring Genetic Networks and Identifying Compound Mode of Action via Expression Profiling. Science 301: 102-105 (2003).